

PART II.—Experiments on the splitting of oils by means of Castor Seed Lipase.

by J. J. Sudborough, H. E. Watson and P. S. Varma.

I. INTRODUCTION.

The production in India of a crude glycerine capable of readily yielding a dynamite glycerine on distillation attracted a good deal of notice during the course of the war and our attention having been drawn to the subject by the Munitions Board we carried out the experiments described in this part in order to determine whether it would not be possible to accomplish this object by means of the well known castor seed ferment (see Part I).

There are five methods of producing glycerine from natural fixed oils and fats :--

1. Superheated steam with a little lime in autoclaves, the product is known as saponification glycerine.
2. Dilute sulphuric acid under pressure gives crude distillation glycerine.
3. The Twitchell reagent, consisting of a complex sulphonic acid derived from oleic acid and an aromatic hydrocarbon, or the later more rapid "Kontakt" reagent (see Part IV).
4. Caustic soda in the manufacture of soap, when glycerine is present in the so called spent lyes and is now generally recovered: the product is known as crude soap lye glycerine.
5. Castor seed ferment, when the product is known as crude fermentation glycerine.

It appears to us that the last possesses many advantages from the point of view of production in India provided some of the defects could be overcome.

The advantages are:—1. The use of simple plant which could be made in the country *e. g.* mainly wooden vats. 2. The avoidance of the use of expensive chemicals. Twitchell's or the "Kontakt" ferment would have to be imported and substances like sulphuric acid are more expensive in India than in Europe or America. The only chemicals necessary would be the small amounts of activators, small amounts of sulphuric acid and the materials necessary for refining the dilute glycerine. 3. The fact that the fatty acids formed should find a ready sale for candle and soap making, especially as in the latter case the soap can be

manufactured by using the comparatively cheap alkali, carbonate of soda, whereas in the ordinary process of soap manufacture the more expensive caustic soda is essential. This is sometimes made from the carbonate in the soap factory, but even this entails extra cost. 4. The normal atmospheric temperature in many places is practically that required for rapid hydrolysis so that no artificial heating is required.

The drawbacks of the fermentation process are:—1. The comparatively large middle layer formed at the end of the operation, when glycerine liquor is separated from the fatty acids. This middle layer retains an appreciable portion of the glycerine liquor and the removal of the latter is difficult. 2. The impure nature of the crude glycerine obtained. This is largely due to the soluble proteins present in the castor seeds; these are ultimately found in the crude glycerine and render the production of dynamite glycerine by distillation a difficult operation.

As pointed out in Part I (p. 219) attempts have been made to overcome these difficulties by using preparations made from the seeds in places of the seeds themselves. All the methods adopted have had as their object the removal of protein matter, other than the lipase ferment, from the seeds in order (1) to reduce the middle layer to a minimum and (2) to obtain a crude glycerine as free from protein matter as possible.

Of the numerous preparations described in Part I, section 1 the only ones which appear to have possibilities for employment on the commercial scale are:—

1. The German process due to Hoyer (p. 221).
2. The French process due to Nicloux (p. 219).
3. The Japanese process due to Tanaka (p. 222).

We have carried out a number of experiments using these three types of preparation, more especially the first and second and our experiments lead to the conclusion that the French method or some modification of it is the simplest to work.

II. GENERAL METHOD OF PROCEDURE.

In most of the small scale experiments the commercial oil—cottonseed oil but in a few cases ground nut oil—the acid and saponification values of which has been previously determined, was mixed with the ferment—either the crushed decorticated castor seeds or some preparation obtained from the crushed seeds—in the proportion of 100 parts by weight of oil to 4 parts by

weight of seed—and the water and activator subsequently added, usually in the proportion of 36—50 parts by weight of 0.0277 N acetic acid. In order to obtain a good, permanent emulsion the mixture was agitated automatically by a simple stirrer consisting of two glass rods fixed in a holder and driven by a hot air motor. The stirring arrangements were so arranged that five experiments could be conducted at the same time and each stirrer worked by the same driving band. In some case the stirring was continued during the whole period of the experiment, in others after four hours stirring the mixture was kept at rest and it was found that the emulsion remained permanent.

The method adopted for estimating the percentage of oil hydrolysed was somewhat simpler, although perhaps a little less accurate, than that described by Hoyer (p. 236) and consisted in removing at any given time a weighed quantity of the emulsion, usually about 5-10 grams, dissolving this in 90 per cent. alcohol, making up to 200 or 250 c.c. and titrating an aliquot part against standard sodium hydroxide solution using phenol-phthalein as indicator.

From the total weight of the emulsion and also the proportion of oil present, the quantity of oil taken was readily calculated, and after subtracting the amount of alkali required for any free acid originally present in the oil and for the acetic acid used as activator, the amount of alkali required to neutralise the fatty acids liberated by the hydrolysis of the oil was ascertained; and knowing the original saponification value of the oil, the percentage of the oil hydrolysed in the given time was readily calculated.

Thus in one experiment using an oil with an acid value* 2.4 and a saponification value* 32.77, after 4 hours 6.40 grams of emulsion were removed and made up to 250 c. c.; 25 c. c. of this solution required 11.16 c. c. of 0.1N. caustic soda for neutralisation. The 6.40 grams of emulsion were equal to 4.636 grams of oil, hence one gram of oil is neutralised by 24.06 c. c. of the standard alkali.

The alkali was used in neutralising any free acids originally present, the acetic acid used as accelerator† and the free fatty acids produced during the reaction; as the original acidity

* See foot note p. 245.

† This value for 1 gram of emulsion is so small that it may be neglected.

of the oil was 2.42, the actual amount of alkali required to neutralise the acids produced from one gram of oil is $24.06 - 2.421 = 21.64$ and hence the per cent of hydrolysis = $\frac{21.64 \times 100}{32.77 - 2.42} = 71.2$.

A slight inaccuracy is due to the fact that during the course of the experiments, some of which lasted 72 or 96 hours, a certain amount of water was lost by evaporation, and hence towards the end of the experiment the composition of the emulsion would have altered somewhat, quite apart from the hydrolysis which had taken place. In one experiment using 200 grams of oil, 80 c.c. of dilute acetic acid and 26.3 grams of Nicloux' ferment at $23.5 - 26.5^\circ$ a loss of 7.0 gram had taken place after 24 hours continuous stirring. In a similar experiment the loss was 8 grams.

Two parallel experiments were conducted in order to determine the error thus introduced. In the one experiment the hydrolysis was determined by the method just described and in the other by removing a portion of the mixture, breaking the emulsion by means of sulphuric acid, washing the oily layer with water, drying it and then titrating the fatty acids in a given weight by means of standard sodium hydroxide. For the first 18 hours the results were practically identical and even after 48 hours the former method gave 93.4 per cent. hydrolysis and the latter 91.7 per cent.

III. SOME PRELIMINARY EXPERIMENTS.

a) Action of dilute acetic acid in the absence of castor ferment. As the statement had been made to us that appreciable hydrolysis of groundnut oil was produced by dilute acetic acid in the course of half an hour and in the absence of castor seed ferment, we carried out the following experiments:—

(1) A mixture of 50 grams of refined ground nut oil, free from acidity, was stirred with 20 c.c. of dilute acetic acid (1.66 grams per litre) and the mixture titrated after 5 hours at $24 - 27^\circ$. The amount of fatty acid corresponded with only 0.2 c.c. of 0.1 N caustic soda solution (2) A similar experiment made with water required 0.15 c.c. of 0.1 alkali. (3) A similar experiment made with commercial groundnut oil and water (50 : 20) required 39.2 c.c. of 0.1 N alkali for the free acids present and (4) a sample of the same oil stirred with dilute acetic acid (50 oil : 20 dil. acid) required 44.9 and this less the 5.7 necessary for the acetic acid gives the same result as experiment 3, viz. 39.2 c.c. These results show that dilute acetic acid does not produce any

recognisable hydrolysis in the course of 5 hours with either acid-free groundnut oil or the commercial oil containing free fatty acid.

b) As already stated several experiments were carried out at the same time and so arranged that each stirrer was driven by the same band; a series of experiments was made by taking five oil ferment mixtures of the same composition and stirring all at the same time and ascertaining whether the percentages hydrolysed were at all concordant.

The results given in Table I show quite good agreement. Each mixture contained 100 grams of castor oil (with an acid* value 2.42 and a saponification value 32.77), 4 grams of crushed castor seed and 36 c.c. of dilute acetic acid (1.66 grams per litre).

Table No. I. Experiment No. 5.

	1.	4.	10.	18.	28.	Time in hours.
Beaker No 1 ..	40.8.	71.2	85.1.	95.5.	99.2.	% of oil hydrolysed
.. 2 ..	40.5.	70.9.	85.1.	94.9.	98.9.	..
.. 3 ..	45.1.	71.0.	86.3.	94.9.	98.9.	..
.. 4 ..	52.1.	70.9.	86.8.	94.7.	98.5.	..
.. 5 ..	53.9.	71.8.	86.4.	94.8.	98.8.	..

A second series of experiments was made with castor, cotton seed and groundnut oils in order to show that control experiments made after an interval of several days gave concordant results.

Table No. II gives the results of these experiments.

Table No. III gives the results of a number of experiments made with different oils using crushed castor-seed as catalyst and either acetic or sulphuric acid as activator.

*In all the experiments the acid value indicates the No. of c.c. of 0.1 N alkali required to neutralise the free acids present in one gram of the oil. In a similar manner the saponification number indicates the number of c.c. of 0.1 N alkali required to react with the total acid (free acid + acid combined as esters) in 1 gram of oil.

TABLE II.

Each experiment was made with 100 grams. of Oil, 4 grams of Crushed Castor Seed
and 36 c. c. of acetic acid solution.

No. of Experiment	Oil used	Acid value of oil	Saponification value of oil	Per cent of oil hydrolyed after						Date of Experiment	Temperature
				1	4	10	18	28	48 hours		
6	Castor	0.86	32.05	26.9	61.0	77.4	85.4	90.9	94.9	June 4th	25-27°
7	"	"	"	31.9	69.9	...	88.9	93.0	96.1	" 20th	27-30°
8	Cotton Seed	0.20	35.67	21.1	55.2	76.2	85.9	89.9	95.2	June 4th	25-27°
9	"	"	"	19.7	54.1	74.2	84.4	87.3	92.5	" 27th	23-26°
10	Ground Nut	9.33	35.33	24.5	51.6	72.8	77.6	85.9	90.4	July 6th	23-26°
11	"	"	"	24.3	52.0	74.5	78.1	87.9	90.8	" 27th	23-26°

TABLE III.
Preliminary Experiments.

No. of Exp't.	Oil used	a	b	c	d	e	f	g	Per cent of hydrolysis after						REMARKS
									1	4	10	18	28	48 hours	
12	Ground-nut	100	Nil	33.63	4	36	Nil	Nil	0.6	1.0	1.7	3.0	5.5	7.3	
13	"	"	0.74	33.77	4	36	"	"	0.8	1.4	2.1	3.5	6.1	8.0	
14	"	"	0.33	35.33	4	36	"	"	0.597	49.6	62.1	82.1	81.1	90.3	94.4
15	Castor	20	2.23	32.70	0.8	7.2	acetic	0.0120	24.8	50.8	68.5	77.9	79.3	...	
16	"	"	"	"	"	"	"	"	32.5	60.0	77.9	78.3	82.2	...	
17	"	"	"	"	"	"	"	"	23.1	36.7	45.5	47.7	63.1	...	Stirring more rapid. The crushed castor seed had been kept for two days before being used.
18	"	"	"	"	"	"	"	"	24.3	36.9	67.7	67.9	73.3	...	A smaller variety of seed used.
19	"	"	"	"	"	"	"	"	28.7	55.7	72.0	76.0	78.6	...	The seed was ground with some of the acetic acid solution for 45 minutes before adding the oil and the remainder of the acetic acid solution.
20	"	"	2.43	32.78	"	"	"	"	27.9	61.2	65.3	77.8	84.4	...	
21	"	100	0.86	32.05	4	36	"	0.0597	26.9	61.0	77.4	85.4	90.9	94.9	
22	Cotton-seed	1000	0.02	34.23	40	360	sulphuric	0.424	7.9	29.3	55.3	70.5	83.4	90.1	
23	"	100	0.20	35.67	4	36	acetic	0.0597	21.1	55.2	76.2	85.9	89.9	95.2	

- a Quantities of oil in grams
b Acid value of oil expressed in no of c. c. of 0.1 N. alkali required to neutralise one gram of oil
c Saponification value expressed in no of c. c. of 0.1 N alkali required to neutralise and saponify 1 gram of oil
d Weight of castor seed as ferment
e Water added in grams
f Activator used
g Amount of activator expressed in grams

IV. EXPERIMENTS ON HOYER'S METHOD.

In this method (cf. p. 221) the ferment is prepared by grinding the decorticated castor seed with 10 times its weight of water, removing all coarse particles by filtration through cloth and then allowing the milky emulsion to remain at rest for several days. During this time fermentation occurs, a foetid odour, resembling that of decayed cheese, is developed, bubbles of gas escape and a scum rises to the surface; at the same time a white flocculent deposit settles at the bottom and the intermediate liquid becomes nearly transparent. The upper layer is removed, freed as far as possible from water, but not washed, and is used as the ferment, either with or without an accelerator.

Table IV gives the results of a number of experiments carried out with this process. The fermenting liquid was kept for different periods of time and the top layer removed and used for hydrolysing the oil. Experiments were also made with the bottom layer from certain preparations. As the ferment itself shows distinct acidity *e.g.* in some experiments 1 gram of the ferment neutralised 15.4 c. c. of 0.1 N alkali, this acidity was taken into consideration in determining the amount of oil hydrolysed after given intervals of time.

The ferment used in experiments 24 to 35 was prepared by grinding the seeds and water in a pestle and mortar by hand and it was found that the amount of top ferment obtained from 50 grams of seed varied only between 49–52 grams and that the amount of deposit at the bottom of the vessel was very small. In each of these experiments 4 grams of ferment corresponding with 4 grams of seed was used for 100 grams of oil. The results of these experiments indicate that the time during which the liquid is allowed to ferment has very little effect upon the activity of the ferment within the limits 72 to 120 hours, and that the activity is practically identical with that of the same weight of seed. (Compare experiments 15 and 16 with 24 to 29) By taking a larger proportion of the ferment it is possible to increase the rate of hydrolysis (comp. expt. 49). In experiments 34 and 35 acetic acid was not added and the results of hydrolysis are very poor, indicating the need of a co-enzyme when Hoyer's preparation is used.

Experiments 36 to 42 were made with undecorticated seeds. In the preparation of the ferment an appreciable amount of a deposit was obtained in addition to the top layer, and separate experiments were made with the top and bottom layers, but with both the amount of hydrolysis was negligible.

Table IV.

Experiments using Hoyer's method.

In each experiment 100 grams of oil and 36 c. c. of water were used.

No. of Experiment	Temp in degrees c	Oil used	b	c	d	f	g	Per cent of Hydrolysis						Remarks
								1	4	10	18	28	48 hrs	
24	25-27.5	Castor	0.86	32.05	4	Acetic	0.0597	18.0	40.8	59.3	69.8	79.2	—	Decorticated. 72 hours.*
25	"	"	"	"	"	"	"	18.2	40.7	56.0	70.2	75.9	—	
26	"	"	"	"	"	"	"	26.3	45.6	63.1	71.5	79.6	—	
27	"	"	"	"	"	"	"	25.3	43.5	59.4	71.7	80.1	—	
28	"	"	"	"	"	"	"	17.9	16.0	65.6	74.8	82.3	—	
29	"	"	"	"	"	"	"	20.1	46.7	64.6	75.7	82.3	—	
30	"	"	"	"	"	"	"	13.0	30.3	52.1	62.2	70.9	—	
31	"	"	"	"	"	"	"	12.6	30.8	50.0	60.9	69.0	—	
32	"	"	"	"	"	"	"	20.5	40.5	57.0	67.7	74.3	—	
33	"	"	"	"	"	"	"	19.9	40.5	56.8	66.8	73.6	—	
34	23-26.5	"	2.42	32.77	"	Nil	Nil	1.7	2.4	3.3	3.4	4.2	—	
35	"	"	"	"	"	"	"	0.8	1.9	3.0	3.1	4.0	—	
36	25-26	"	1.04	32.95	1.5	acetic	0.0597	1.0	0.4	2.4	—	—	—	
37	"	"	"	"	"	Nil	Nil	1.0	1.2	0.36	—	—	—	
38	"	"	"	"	1.5	acetic	0.0597	1.1	1.3	2.7	—	—	—	
39	"	"	"	"	"	Nil	Nil	0.8	0.5	0.2	—	—	—	
40	25-27	Ground nut	0.66	33.76	3.0 T	acetic	0.0597	5.2	16.2	25.9	32.3	—	—	
41	"	"	"	"	"	Nil	Nil	4.8	5.1	6.0	6.2	—	—	
42	"	"	"	"	3.0 B	acetic	0.0597	4.5	7.9	9.2	10.5	—	—	
43	"	"	"	"	1.5 T	"	"	6.6	14.7	25.6	32.6	37.9	45.6	
44	"	"	"	"	1.5 B	"	"	6.3	16.7	29.0	32.8	42.7	52.5	
45	"	"	"	"	1.5 T	"	"	2.0	2.9	3.9	4.0	5.2	6.3	
46	"	"	"	"	1.5 B	"	"	2.4	4.8	10.9	21.4	33.7	50.2	
47	21-26	"	"	"	1.5 T	"	"	4.2	5.0	8.6	13.8	26.6	—	
48	"	"	"	"	1.5 B	"	"	1.8	2.8	5.4	8.1	9.5	20.3	
49	25-27	Castor	2.42	32.77	7.6	"	"	30.2	59.1	75.3	87.6	94.8	—	

T= top layer.

B= bottom layer.

d= Weight of ferment and corresponds with 4 grams of castor seed, except in Expts. 40, 41, 42, 49 where a larger proportion of ferment was used.

*The numbers indicate the length of time the liquid was allowed to ferment before the scum was removed.

Attempts were made to grind the seeds and water in some type of mill, as on a large scale it would not be feasible to grind by hand in a pestle and mortar. Both a ball mill and also a small edge runner mill were used. In all cases it was found that the amount of residue left on the cloth in the preliminary filtering was large, and that during the formation of the ferment an appreciable bottom layer was obtained. The results of the hydrolysis are recorded in Table IV under experiments 43 to 48, and show that both the bottom and top layers have lipolytic activities, but the amounts are small for the quantities of ferment used. In all these cases the amount of top layer was extremely small; the actual values are:—

Experiment.	Seed.	Residue on cloth.	Top layer.	Bottom Layer.
43 and 44	175	45	45	60
45 and 46	175	45	50	49
47 and 48	100	21	22	30

V. EXPERIMENTS BASED ON TANAKA'S METHOD.

In Tanaka's original description (cf. p. 222) ether is recommended as the solvent for the removal of oil from the crushed castor seeds. Our experiments have been made with toluene.

50 grams of decorticated castor seeds were ground in a mortar and the cake digested several times with toluene for two days at the ordinary temperature (23-26°). The product was then filtered through mull cloth in order to remove coarse particles and finally through filter paper to remove the fine particles. The coarse particles were washed with toluene, dried, and reground repeatedly until nearly all passed through a 60-mesh sieve. The powder so obtained was mixed with the powder collected on the filter paper and the whole dried at a temperature not exceeding 30°, when 14 grams of dry powder were obtained, together with 3.8 grams of coarse powder.

Exp. 50—100 grams of castor oil, 36 c. c. of dilute acetic acid solution and 1.2 grams of the fine powder (=4 grams of crushed seed) were mixed and stirred and the following results were obtained:—

Per cent of oil hydrolysed	2.4	5.0	8.9	11.3	11.6.
Time in hours.	1.	4.	10.	18.	28.

Expt. 51. Similar to No. 50 except that 1.2 grams of the coarse powder was used.

Per cent. of oil hydrolysed.	1.7.	2.9.	5.4.	7.4.	8.3.
Time in hours	1.	4.	10.	18.	28.

Expt. 52. Similar to No. 50 but using a new preparation of the fine powder.

Per cent. of oil hydrolysed	4.6.	12.6.	23.7.	33.9.	43.7.
Time in hours.	1.	4.	10.	18.	28.

100 grams of the powder as used in expt. 52 were triturated with 650 c. c. of 0.1 N acetic acid at 30—34° for half an hour. The milky liquid was then filtered, the residue washed with water and dried at a temperature not exceeding 30°.

Expt. 53. 100 grams of castor oil, 36 c. c. of water and 1.2 grams of this powder were used.

Per cent of oil hydrolysed.	0.8	0.9	1.0	1.3.
Time in hours.	1.	4.	10.	18.

Expt. 54. Similar to No. 53 except that 0.2 gram of manganese sulphate was added in order to see whether it produced any increase on the amount of hydrolysis.

Per cent. of oil hydrolysed.	0.95.	1.2.	1.4.	1.6.
Time in hours.	1.	4.	10.	18.

As the method of extraction with a volatile solvent is tedious and as the experiments made with the French process (section VI) gave promising results the investigation of the Japanese method, in order to determine the conditions for optimum yields, was not continued.

VI. EXPERIMENTS ON NICLOUX' METHOD.

The method adopted for preparing the ferment on a small scale was usually similar to that described by Nicloux. Decorticated castor seeds were ground for 30 minutes in an edge runner mill with twice their weight of cotton seed oil, the pulpy mass so obtained was then gently pressed through a 40 mesh sieve and finally through fine mull cloth. The wire sieve retained the coarse aleurone particles, and the cloth the fine aleurone particles together with some ferment. The turbid, oily liquid, which passed through the cloth, was used as the hydrolysing agent.

Table V gives the results of a number of experiments made with ground-nut oil and cotton-seed oil using acetic acid as activator. Table VI gives results for cotton seed oil using sulphuric acid as activator and Table VII results with manganous sulphate as the activator. Table VIII records experiments made at different temperatures using either acetic acid or sulphuric acid as co-enzyme.

TABLE V.
Experiments using Nicloux' method
with acetic acid as accelerator.

No. of Exp't	Temp in degrees C.	Oil used	a	b	c	d	e	g	Per cent of hydrolysis after						Remarks
									1	4	10	18	28	48 Hrs.	
55	25-28	Ground nut	100	1.04	32.95	7.0	36	0.0597	12.8	34.3	51.1	58.7	63.8	72.0	1
56	24-28	"	"	"	"	2.3*	"	"	12.5	32.3	57.4	50.0	60.6	66.8	1
57	24-28.5	"	"	0.66	33.80	10.5	"	"	3.7	5.2	22.0	37.9	49.9	64.2	2
58	24-26	"	"	0.65	33.71	7.0	"	"	3.4	9.0	22.5	37.6	45.1	—	1
59	"	"	"	"	"	10.0	"	"	7.3	23.5	47.7	66.6	73.8	—	1
60	"	"	"	"	"	14.0	"	"	—	—	—	75.3	77.5	—	1
61	24-26	Cotton Seed	700	0.04	34.11	70.0	252	0.418	—	49.4	57.0	71.8	78.6	85.0	1
62	27-30	"	100	0.03	34.40	13.1	40	0.0664	36.5	52.1	83.2	98.1	—	—	3
63	"	"	"	"	"	"	"	"	35.7	51.4	82.2	96.2	—	—	3
64	25-28	"	"	"	"	"	"	"	29.0	54.0	71.6	83.4	—	—	4

- Remarks 1 Ferment prepared by grinding in edge runner mill, pressing through wire sieve and then through cloth.
 2 Stale ferment, used 4th day after preparation
 3 Ferment prepared by grinding in edge runner mill and then centrifuging in a tube centrifuge and taking the upper turbid layer as ferment
 4 Similar to 3 but product twice centrifuged in a tube centrifuge and the upper oily layer used as the ferment.
 d Weight of oily ferment in grams, 7 grams corresponding with 4 grams of seed.
 * 23 grams of fine residue retained by the cloth in straining the ferment.

TABLE VI.

Experiments with Nieloux' method using Sulphuric acid as accelerator at 22--25°
 The oil used was cotton seed oil with an acid value 0.04 and a saponification value 34.41

No. of Experiment	Oil taken in grams	Weight of ferment in grams	Volume of liquid	Weight of sulphuric acid in grams	Percent of Hydrolysis after						Remarks on preparation of the ferment.
					1	4	10	18	26	48 hours	
65	100	10.5	36	0.05	0.92	0.85	0.84	0.89			Same as No. 56
66	"	"	"	"	0.87	1.1	1.1	1.4			
67	"	"	"	0.025	2.8	16.4	27.6	40.5	43.1	52.2	"
68	"	"	"	"	2.0	14.4	23.0	37.9	42.2	51.5	"
69	"	"	"	0.0125	20.6	48.7	68.4	78.7	82.5	91.6	"
70	"	"	"	"	23.3	49.2	69.1	80.1	83.6	92.2	"
71	"	"	"	"	29.1	45.1	...	70.6	82.5	85.2	"
72	"	"	"	"	19.9	45.8	...	76.3	84.3	88.2	} A new ferment preparation used. Stirring was discontinued after 4 hours.
73	"	"	"	"	3.2	19.3	43.0	59.6	62.6	71.9	
74	"	"	"	"	9.9	31.8	53.4	66.5	74.1	77.6	} Used cake obtained by cold pressing seed and ground this with its own weight of cotton seed oil. From 67 grams cake only 80 grams of ferment was obtained.
75	"	"	"	"	1.0	1.5	1.8	2.6	3.4	...	
76	"	"	"	"	0.7	1.4	1.5	1.7	2.0	...	} Undecorticated seed used for preparing ferment.
77	"	"	"	0.005	8.5	17.5	27.6	34.0	37.3	49.3	
78	"	"	"	"	8.6	15.1	28.3	34.2	37.8	49.7	} Same as No. 56
79	"	"	"	0.0125	7.3	28.0	43.5	54.4	64.9	67.1	
80	"	"	"	"	4.1	16.9	34.7	44.7	48.6	58.2	} As above only less cotton seed oil used for grinding cake (1:1)
81	"	"	"	"	17.8	40.9	60.4	71.7	
82	"	"	"	"	17.5	39.5	56.8	64.6	} As in Experiment 56
83	1768	185	636	0.1951	10.4	49.6	63.9	78.3	
84	"	"	"	"	20.3	57.9	75.3	82.0	} " "
85	1000	105	360	0.125	7.6	31.8	51.7	62.1	69.8	78.9	

253

* acid value of the sample of oil used was 0.2 and the saponification value 35.7.

TABLE VII.

Experiments with Nieloux' Method using Manganous Sulphate as accelerator.

In each experiment 100 grams of cotton seed oil, 13.1 gram of ferment and 40 cc. of water were used.

No. of Exp't.	Temp. in degrees C	Acid value of oil	Saponification value of oil	Weight of Mang. Sulphate	Per cent of hydrolysis after						REMARKS.
					1	4	8	10	18	28 hrs.	
86	24/27	0.02	34.40	0.20	0.9	1.5	—	76.9	84.1	89.8	A. in Exp 56 " " " " " " " " " In experiments 92 to 99 the ferment, the activator and 5 per cent of the water were stirred for a short time then kept for 10—18 hours before the oil was added.
87	"	"	"	"	1.2	5.6	—	78.1	86.0	95.1	
88	23/26	"	"	0.25	0.6	1.9	30.8	74.5	83.3	93.9	
89	"	"	"	0.15	0.8	1.7	48.0	75.7	85.9	95.8	
90	"	"	"	0.10	0.6	1.1	2.8	80.3	60.0	79.7	
91	"	"	"	0.05	Nil	Nil	Nil	Nil	Nil	Nil	
92	"	"	"	0.20	35.2	54.9	—	—	—	—	
93	24/29	"	"	0.20	—	—	55.4	66.8	—	—	
94	13/24	"	34.15	"	—	55.9	69.0	—	85.1	—	
95	"	"	"	"	23.6	47.1	67.4	76.5	82.0*	—	Ferment prepared by removing coarse particles by basket centrifuge using fine cloth. Same as No. 90 and 91 only using somewhat coarser cloth so that ferment was somewhat more turbid.
96	"	"	"	"	15.7	30.6	—	52.9	55.8	—	
97	"	"	"	"	16.5	31.4	—	53.8	58.1	—	
98	"	"	"	"	24.5	49.9	68.4	77.7	83.1	—	
99	"	"	"	"	20.6	50.7	68.2	78.7	83.1	—	

*In 2 1/2 hours

TABLE VIII.

Experiments with Nicloux' ferment at different temperature.

In each experiment 100 grams of cotton seed oil were used. The acid value of the oil was 0.03 and the saponification value 34.10.

No. of Experi.	Temperature	Weight of ferment in grams	Volume of water in c.c.	Accelerator	Weight of acclr. in grams	Per cent hydrolysis after						Remarks
						1	4	10	18	28	48 hours	
100	20—27	10.5	36	Ac	0.0597	11.5	29.4	52.7	62.0	—	—	Ferment prepared as in experiment No. 56
101	"	"	"	S	0.0125	3.1	22.7	56.1	66.0	—	—	
102	23—29.5	15.7	54	Ac	0.0895	19.0	49.0	70.8	79.7	86.4	97.9	"
103	"	"	"	S	0.0182	6.5	31.2	54.7	69.4	75.7	84.7	"
104	24—30	"	"	Ac	0.0895	23.0	53.9	74.0	83.4	90.6	99.0	"
105	"	"	"	S	0.0182	7.6	34.8	61.4	74.1	80.1	90.2	"
106	28—30.5	"	"	Ac	0.0895	39.7	68.6	87.7	96.0	101.0	—	"
107	"	"	"	S	0.0182	15.9	36.3	58.7	68.6	78.3	88.4	"
108	"	10.5	36	Ac	0.0597	25.4	57.1	74.0	86.9	91.8	97.0	"
109	"	"	"	S	0.01212	18.7	48.7	67.7	76.9	85.0	90.2	"
110	28—31	13.1	45	Ac	0.0895	31.2	64.2	81.5	90.3	97.7	100.7	"
111	"	"	"	S	0.01515	21.5	52.9	72.6	82.2	89.2	97.4	"
112	32—33	4	36	Ac	0.0597	19.1	50.7	80.7	96.5	98.1	98.6	} used ground castor seed
113	"	"	36	S	0.0424	13.2	33.6	45.5	67.7	76.9	80.9	
114	33—35	15.7	54	Ac	0.0895	8.0	15.7	19.3	21.6	23.2	—	"
115	"	"	"	S	0.0182	4.4	8.0	12.8	13.2	10.8	—	"
116	34—36	"	"	Ac	0.0895	4.4	6.9	8.6	10.2	10.8	—	"
117	"	"	"	S	0.0182	3.1	5.1	7.6	8.5	9.6	—	"
118	37—42	"	"	Ac	0.0895	3.3	3.9	4.2	4.6	4.9	—	"
119	"	"	"	S	0.0182	1.5	1.5	2.5	2.6	2.7	—	"

S = Sulphuric acid.

Ac—Acetic acid.

*Conclusions drawn from the experiments included in
Tables V to VIII.*

1. A comparison of experiment 55 with experiment 61 indicates that the groundnut oil is not hydrolysed so readily as cotton seed oil under similar conditions. Compare also results in Table II.

2. The results show that with cotton seed oil 95—98 per cent hydrolysis can be obtained within 48 hours by using preparations of the type of those described by Nicloux. Such preparations can be made by any one of the three processes:—(a) grinding castor seed with twice its weight of refined cotton seed oil and then pressing through wire gauze and finally through cloth to remove solid particles (compare experiments 55-61) (b) grinding the seed with the cotton seed oil and separating the coarse particles by means of a tube centrifuge and using the turbid oily layer (cf. experiments 62 and 63; (c) grinding the seed as in (a) or (b) and removing the coarse particles by means of a centrifuge in which the wire gauze of the basket is covered on the inside with a layer of longcloth. The turbid oil which collects in the outer cage is highly active (cf. expt 96—99.)

3. It is highly advisable to use ferment which is freshly prepared, as experiment No. 57 indicates that after 3 days the activity is diminished.

4. An appreciable difference is encountered when undecorticated seeds are used for preparing the ferment, as the saponification proceeds much more slowly than when using the equivalent quantity of ferment from decorticated seed. (Expts. 75 and 76).

5. When cold pressed castor cake is used as the source of the ferment the quantity of the ferment emulsion necessary to produce rapid hydrolysis is also much greater (Experiments 73 and 74). The cake was several days old before it was used.

6. The results confirm Hoyer's statement that small variations in the total amount of sulphuric acid present with a given weight of oil or of ferment affect the rate of hydrolysis in a marked manner (experiments 65 to 78). As it is difficult to obtain a ferment preparation which always has the same lipase contents, it is clear that working on a commercial scale and using sulphuric acid as activator it would be necessary to make small scale tests with each batch of ferment in order to ascertain the amount of sulphuric acid to be added to obtain the optimum effect. Acetic

acid has the great advantage that the maximum rate of hydrolysis can be attained within relatively wide limits of concentration of the acid. It has, however, the drawback that it is not readily eliminated from the crude glycerine.

7. Manganous sulphate is an excellent activator, and when used in the proportion of 0.2 parts of the crystallised sulphate per 100 parts of oil and 4 parts of crushed seed, or the equivalent quantity of ferment preparation, produces 90—95 per cent. hydrolysis within 48 hours. (Experiments 86 to 89). A reduction however of the amount of activator to 0.05 parts per 100 of oil reduces the hydrolysis practically to zero.

8. When manganous sulphate is used the hydrolysis proceeds very slowly within the first four hours, but then proceeds rapidly. If, however, the ferment, manganous sulphate and a portion of the water are stirred for a short time, then left for 10—18 hours and finally added to the oil and the remainder of the water, the hydrolysis proceeds rapidly from the beginning (experiments 92 to 99) although after 48 hours or even 28 hours the amount of oil hydrolysed does not differ materially from what is obtained when this preliminary treatment is not adopted.

9. Continual stirring is not necessary, using acetic acid a preliminary stirring for four hours is all that is required to obtain a stable emulsion which remains permanent for 48 hours. If, however, the manganous sulphate, ferment preparation and part of the water are not first kept in contact for some time the period of stirring required is much longer (*cf.* experiments 121 to 123).

10. A temperature of 30 or 31° appears to give the best results, the rate of hydrolysis is much reduced when a temperature approaching 36° is used and become almost negligible at 40--42°. (Experiments 100 to 119).

VII. LARGE SCALE EXPERIMENTS.

As the results of the experiments on the small scale we came to the conclusion that the French method would be the most convenient to work on a larger scale and therefore several experiments were made with quantities of about 100 kg. of cotton seed oil using either acetic acid or manganous sulphate as activator. The experiments were carried out in a jacketted enamelled iron pan provided with a geared stirring arrangement.

In order to obtain a crude glycerine as free from protein matter as possible the original oil was steamed for about 5 hours, allowed to settle and the clear oil syphoned off.

The general method of preparing the ferment was as follows:—5 kg. of decorticated castor seeds were ground with 10 kg. of cotton seed oil in an edge runner mill for 35 minutes when the mixture formed a pulpy mass. This was then filtered through a 40 mesh sieve and finally squeezed through cloth and about 12 kg. of the turbid oil ferment were obtained. About 1 kg. of coarse residues remained on the sieve and 2 kg. of fine residues on the cloth.

In experiment No. 123 the solid particles were removed by placing the pasty mass in a basket centrifuge lined with mull cloth and centrifuging for 40–45 minutes. About 11 kg. of ferment and 4 kg. of residue were obtained.

Experiment No. 120. The mixture used consisted of 32.7 kg. of cotton seed oil (with an acid value=0.10 and a saponification value=34.50*), 13.6 kg. of water containing 23.9 grams of glacial acetic acid, and 4.2 kg. of ferment. The whole was stirred for 11.5 hours and kept at a temperature of 26–29° and then the stirring was stopped. After 4 hours 66.1 per cent. of oil was hydrolysed, after 11.5 hours 89.2 and after 22 hours 93.7. At the end of 28 hours the mixture was heated to 80° and 84 grams of commercial concentrated sulphuric acid and 42 grams of water added, the whole stirred for a few minutes and then allowed to settle. The upper layer of fatty acids was removed and the lower layer of glycerine water run off and the middle layer washed twice with warm water. The weight of fatty acids was 32.6 kg. and of middle layer 2.7 kg. To remove the sulphuric acid the combined glycerine liquor including washings was neutralised by means of finely ground commercial barium carbonate, and the precipitated barium sulphate removed and washed.

An analysis of the glycerine liquor gave the following values:—

Total weight of liquor	...	8.60 kg.
Sp. gr. at 27°	...	1.084
Glycerine content from sp. gr.	...	36.5 per cent.
Acidity	...	0.0.0
Total ash	...	0.30 per cent.
Total solids at 160°	...	1.15 „
Total glycerine in liquor	...	3.10 kg.

* See footnote p. 245.

A portion of the dilute liquid was concentrated under reduced pressure and a brownish yellow concentrated crude glycerine obtained. This gave the following results on analysis:—

Sp. gr. at 27°	...	1.255
Glycerine content from sp. gr.	...	98.0 per cent.
„ by acetin method	...	91.4 „
Acidity	...	0.056
Total ash	...	0.78 per cent.
Total solids at 160°	...	2.58 „

Experiment No. 121. Temperature 24-28°. The following quantities of materials were used: 89 kg. of cottonseed oil (acid value=0.02 saponification value = 34.40), 36.3 kg. of water, 135 grams of finely powdered manganous sulphate and 11.8 kg. of ferment. These were placed in the pan and stirred for 8 hours (noon to 8 p. m), the stirring was then discontinued but next morning the emulsion had broken and the oil and water separated; the stirring was therefore continued for 8 hours on the second day when a permanent emulsion was formed. The percentages of hydrolysis were:—

24 hours 2.4, 48 hours 92.1 and 72 hours 96.0: At the end of 72 hours the mass was heated to 80° and treated with sulphuric acid (140 grams acid and 140 grams of water) and the three layers separated. The fatty acids* weighed 87.3 kg. and the middle layer 4.8 kg. The glycerine liquor was heated to 80-90° and treated with finely divided barium carbonate (ground up with part of the glycerine liquor) until all the sulphate was removed, this required 850 grams of the carbonate. The greater bulk of the clear liquid was removed by decantation and the remainder by means of a filter press, and the barium sulphate was washed twice with water. The following values were obtained:—

Weight of main glycerine liquor	...	18.2 kg.
Sp. gr. at 27°	...	1.074.
Glycerine content from sp. gr.	...	33.7 per cent.
Acidity	...	0.006.
Total ash	...	0.27 per cent.
Total solids at 160°	...	0.95 „
Total glycerine in liquor	...	6.2 kg.

*The fatty acids obtained in this and subsequent experiments were of excellent colour.

An attempt was made to reduce the total solid content by precipitating soluble proteins by means of aluminium sulphate solution. For every 100 c. c. of glycerine liquid 1 c. c. of a 5 per cent. solution of crystallised aluminium sulphate was used and a flocculent precipitate obtained; the mixture was then treated with barium carbonate followed by a little barium hydroxide solution and a product containing only 0.175 per cent. of ash and 0.72 per cent. of total solids was obtained.

Experiment No. 122. This was similar to No. 121 with the exception of using 82 kg. of cotton seed oil and the corresponding amounts of the other materials. The temperature was 22—25.5°. The mixture was stirred for 8 hours and kept at rest over night. It was partially de-emulsified in the morning and the stirring was continued for another 4 hours when a stable emulsion was produced. After 24 hours 41.6 per cent. of the oil was hydrolysed and after 72 hours 99.0 per cent. The subsequent procedure was as in the previous experiment and the following values were obtained:—

Weight of glycerine liquor	...	14.3 kg.
Sp. gr. at 27°	...	1.105.
Glycerine content from sp. gr.	...	42.5 per cent.
Weight of glycerine	...	6.1 kg.
Washings from the middle layer	...	3.7 „
Washings from the barium sulphate	...	4.2 „

The following analytical results were obtained from the three liquids:—

	1.	2.	3.
	Main glycerine liquor.	Middle layer washing.	Barium sulphate washings.
Weight	... 14.3	... 3.7	... 4.2 kg.
Sp. gr. 27°	... 1.105	... 1.061	... 1.042
Glycerine content from sp. gr.	... 42.5	... 25.7	... 18.4 per cent
Acidity	... 0.044	... 0.019	... 0.002
Total ash	... 0.375	... 0.53	... 0.125 per cent.
Total solids at 160°	... 1.15	... 0.90	... 0.20. „
Weight of glycerine from sp. gr.	... 6.1.	... 0.95	... 0.75 kg.

Total glycerine 7.8 kg.

The main glycerine liquor was treated with aluminium sulphate and then with baryta as in experiment 121 and a liquid with the following constants obtained :—

Sp. gr. at 27°	...	1.089.
Glycerine content from sp. gr.	...	36.5 per cent.
Total ash	...	0.095 „
Total solids at 160°	...	0.70 „
Alkalinity	...	0.020

Experiment 123. In this experiment the ferment was prepared by grinding together 5 kg. of decorticated seed and 10 kg. of cotton seed oil in a ball mill for 1.5 hours and then removing all coarse particles by centrifuging for half an hour in an 18" hand centrifugal at 700—800 revolutions per minute and using a fine mull cloth lining for the basket. 10.2 kg. of ferment liquor and 4.0 kg. of residues were obtained.

The quantities of materials used in the experiment were 64 kg. oil, 25.5 kg. of water, 118 grams of finely powdered manganous sulphate and 9.1 kg. of the ferment.

In order to ascertain the amount of activator which would give the optimum effects, small scale experiments were made by mixing 100 grams of oil, 13.1 grams of ferment and 40 c. c. of water with different quantities of manganous sulphate viz. 0.15 grams, 0.175 gr. and 0.20 grams. At the end of 24 hours the per cent. of oil hydrolysed was 86.7, 83.4 and 81.9 per cent. respectively. The proportion of 0.15 grams of activator per 13.1 grams of ferment was therefore selected for the main experiment.

As the two previous experiments (Nos. 121 and 122) had shown that if the materials are all mixed together and agitated a permanent emulsion is only obtained after more than 12 hours stirring the method adopted in the small scale experiments No. 92 to 99 (Table VII) was used. The ferment, manganous sulphate and 3.4 kg. of water were thoroughly mixed by stirring for half an hour and the mixture kept for 18 hours and during this time no separation occurred. The oil and remainder of the water were then added and the whole stirred for the first 4 hours only when the following results were obtained :—4 hours 52.9 and 24 hours 92.0 per cent. of oil hydrolysed. The temperature was 20—24°. After 28 hours the mixture was heated to 80°, 105 grams of sulphuric acid and 105 grams of water added, the mixture stirred for a few minutes and on standing the three

layers separated in the usual manner. The fatty acid layer weighed 63.2 kg. and the middle layer 4.4 kg. The bulk of the glycerine liquor in this middle layer was removed by centrifuging in a basket hydroextractor lined on the inside with mull cloth.

The glycerine was treated with barium carbonate at 70–80° and the resulting liquor mixed with one per cent. of its volume of a 5 per cent. solution of crystallised aluminum sulphate. On being stirred and then allowed to stand an appreciable amount of precipitated organic matter was obtained. The solution was treated with the requisite amount of barium hydroxide to remove all the sulphate.

The main glycerine liquor weighed 15.5 kg. and the liquor obtained by centrifuging and washing the middle layer 3.65 kg. and the combined liquid when purified and concentrated gave 6.5 kg. of crude liquor with the following values:—

Sp. gr. at 23°	...	1.248.
Glycerine content by acetin method...	...	90.0 per cent.
Ash	...	0.37 „
Total solids at 160°	...	1.85 „
Alkalinity	...	0.043

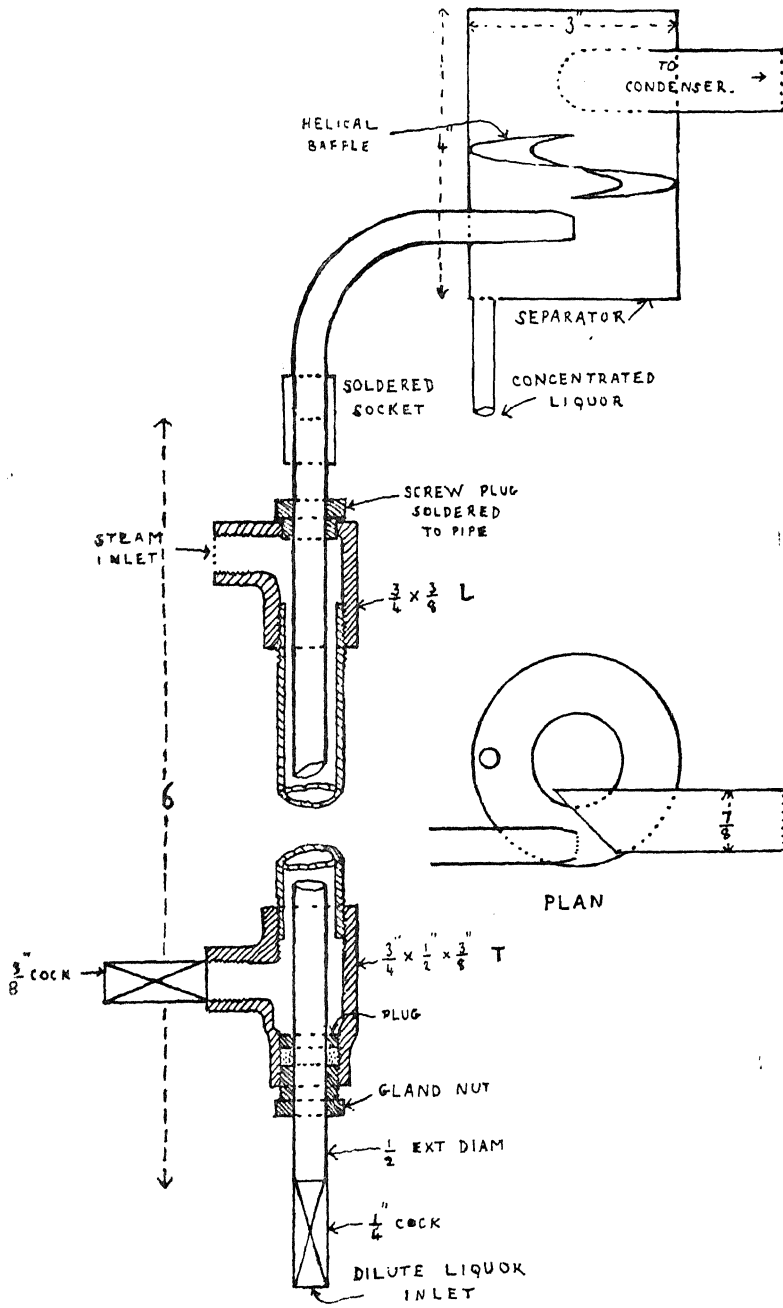
The weight of pure glycerine is therefore 5.9 kg. from the total 64+9.1 kg. of oil employed. This corresponds with an 8.1 per cent. yield of glycerine. The value for cotton seed oil is usually given as 8* per cent.

Concentration of the Crude Glycerine, Analysis and Distillation of the product.

Concentration. In some of the earlier experiments the glycerine liquors were concentrated by heating in glass vessels under reduced pressure, but working with larger quantities we adopted the method of concentrating by means of the small film evaporator described in this Journal Vol. 11. p. 209. The construction is seen from the accompanying figure. With such an evaporator using steam at about 40 lbs. pressure and a vacuum of 80–100 mm. of mercury, it was found possible to concentrate in an hour 7–8 kg. of dilute liquor with a sp. gr. 1.089 to a liquid with a sp. gr. of 1.214.

* Simmons and Appleton (The Hand book of soap manufacture p. 116) give 10 per cent. of an 80 per cent. crude glycerol.

SCALE $4\frac{1}{2}''=1'$



LABORATORY VACUUM FILM EVAPORATOR

Analysis. After removal from the film evaporator a final evaporation in glass under reduced pressure was usually carried out.

Table No. IX gives the analytical data for a sample of concentrated crude Twitchell glycerine made by Messrs. Field Ltd., of Lambeth and for 3 samples of fermentation crude glycerine after concentration.

TABLE No. IX.

	Twitchell Glycerine.	Fermentation (Glycerine.		
		1	2	3
Sp. gr. at 27°	1.245	1.255	1.246	1.235
Glycerine from Sp. gr.	95.0	98.0	95.0	91.1 per cent
Glycerine by acetin method	86.7	91.5	90.0	77.0 „
Total ash	1.05	0.78	0.37	0.45 „
Total solids at 160°	2.35	2.50	1.85	2.20 „
Alkalinity	0.040	...	0.043	nil
Acidity	...	0.056

No. 1 was prepared by using acetic acid as accelerator and without precipitating proteins by aluminium sulphate.

Nos. 2 & 3 were prepared by using manganous sulphate as accelerator and also precipitating proteins with aluminium sulphate and final treatment with barium hydroxide.

These results indicate that the fermentation glycerine is quite as good as, if not better than, the Twitchell glycerine as far as ordinary analysis goes. The ultimate test would, however, be the yield and quality of the refined product produced by distillation with superheated steam under reduced pressure. As we had no suitable plant available for carrying out such distillations, we distilled under reduced pressure in glass vessels by direct heating. For this purpose it was necessary to remove all traces of water before distilling. This was accomplished by heating the crude, concentrated product under reduced pressure (20 mm.) in an oil bath the temperature of which was raised gradually to 200°. In order to avoid loss of glycerine owing to the frothing of the liquid a loose plug of cotton wool was placed in the neck of the vessel.

Two samples of Fermentation glycerine and one of Twitchell glycerine were distilled in this way and it was found

that the Twitchell glycerine gave a superior product. It gave a paler coloured distillate, with not so pronounced a smell and the amount of residue left in the flask was distinctly less.

A sample of the distilled glycerine was submitted to the Government Cordite Factory, Aravankadu, and the report was to the effect that the sample appeared to be quite suitable for nitration but that larger quantities would be required before a completely reliable opinion could be expressed.

We have to thank Mr. K. Panduranga Rao for making several of the analyses mentioned in this part.
